

WHAT IS CLAIMED IS:

1 1. An isolated infectious recombinant respiratory
2 syncytial virus (RSV) comprising a RSV genome or antigenome, a
3 major nucleocapsid (N) protein, a nucleocapsid phosphoprotein
4 (P), a large polymerase protein (L), and a RNA polymerase
5 elongation factor, wherein the recombinant RSV has at least two
6 attenuating mutations, one of the mutations specifying a
7 temperature-sensitive substitution at amino acid Phe₅₂₁, Gln₈₃₁,
8 Met₁₁₆₉, or Tyr₁₃₂₁ in the RSV polymerase gene or a temperature-
9 sensitive nucleotide substitution in the gene-start sequence of
10 gene M2.

1 2. The RSV of claim 1, having at least three
2 attenuating mutations.

1 3. The RSV of claim 1, wherein Leu is substituted at
2 Phe₅₂₁ or Gln₈₃₁.

1 4. The RSV of claim 1, wherein Val is substituted at
2 Met₁₁₆₉.

1 5. The RSV of claim 1, wherein Asn is substituted at
2 Tyr₁₃₂₁.

1 6. The RSV of claim 1, wherein at least two of the
2 mutations are selected from the group consisting of
3 temperature-sensitive substitutions at Phe₅₂₁, Gln₈₃₁, Met₁₁₆₉, and
4 Tyr₁₃₂₁.

1 7. The RSV of claim 6, wherein the
2 temperature-sensitive substitutions are at Phe₅₂₁ and Met₁₁₆₉.

1 8. The RSV of 6, wherein the temperature-sensitive
2 substitutions are at Gln₈₃₁ and Tyr₁₃₂₁.

1 9. The RSV of claim 1, wherein two mutations occur in
2 the codon encoding a temperature-sensitive substitution at
3 Phe₅₂₁, Gln₈₃₁, Met₁₁₆₉, or Tyr₁₃₂₁.

1 10. The RSV of claim 1, formulated in a dose of 10³
2 to 10⁶ PFU of attenuated virus.

1 11. The RSV of claim 1 further comprising a
2 nucleotide modification specifying a phenotype selected from a
3 change in growth characteristics; attenuation,
4 temperature-sensitivity, cold-adaptation, small plaque size,
5 host range restriction, or a change in immunogenicity.

1 12. The RSV of claim 11, wherein a SH, NS1, NS2 or G
2 gene is modified.

1 13. The RSV of claim 12, wherein the SH gene is
2 deleted.

1 14. The RSV of claim 12, wherein the NS2 gene is
2 reciprocally substituted in position for the SH gene.

1 15. The RSV of claim 11, wherein said further
2 nucleotide modification is to a cis-acting regulatory sequence
3 that is modified or rearranged within the RSV genome or
4 antigenome.

1 16. The RSV of claim 15, wherein the cis-acting
2 regulatory sequence is replaced by a heterologous regulatory
3 sequence.

1 17. The RSV of claim 16, wherein the heterologous
2 regulatory sequence is a cis-acting regulatory sequence of a
3 different RSV gene.

1 18. The RSV of claim 11, wherein the nucleotide
2 modification is selected from a termination codon introduced
3 within a selected gene or a change in sequence, position, or

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1 19. The RSV of claim 11, wherein the nucleotide
2 modification comprises an inserted, deleted or altered
3 translational start codon in sequence context within the RSV
4 genome or antigenome.

21. The RSV of claim 11, wherein the RSV genome or antigenome is modified to encode a non-RSV molecule selected from a cytokine, a T-helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of eliciting a protective immune response in a mammalian host.

23. The RSV of claim 22, wherein the PIV gene or gene segment encodes HN or F glycoprotein.

1 25. The RSV of claim 11, further comprising a
2 polynucleotide sequence encoding an immunogenic epitope or
3 protein region of PIV whereby the RSV elicits an immunogenic
4 response to both PIV and RSV antigens.

26. An isolated infectious RSV particle which comprises a recombinant RSV genome or antigenome, a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a

4 large (L) polymerase protein, and a RNA polymerase elongation
5 factor, wherein the genome or antigenome is modified to ablate
6 or modulate expression of a SH, NS1, NS2 or G gene or a
7 cis-acting regulatory sequence.

1 27. The RSV of claim 26, wherein the SH gene is
2 deleted.

1 28. The RSV of claim 26, wherein the NS2 gene is
2 reciprocally substituted in position for the SH gene.

1 29. The RSV of claim 26, wherein the cis-acting
2 regulatory sequence is replaced by a heterologous regulatory
3 sequence.

1 30. The RSV of claim 29, wherein the heterologous
2 regulatory sequence is a cis-acting regulatory sequence of a
3 different RSV gene or of PIV.

1 31. The RSV of claim 26, wherein the RSV genome or
2 antigenome further comprises a gene or gene segment encoding an
3 immunogenic F or G protein region of a different RSV subgroup.

1 32. The RSV of claim 26, wherein the genome or
2 antigenome comprises a chimera of a human RSV sequence and at
3 least one non-human RSV sequence.

1 33. The RSV of claim 26, wherein the genome or
2 antigenome encodes a human RSV in which a selected gene or gene
3 segment is replaced with a counterpart gene or gene segment from
4 a heterologous RSV.

1 34. The RSV of claim 33, wherein the selected gene is
2 NS1 or NS2 and the counterpart gene is N.

1 35. The RSV of claim 26, wherein the RSV genome or
2 antigenome further comprises a nucleotide sequence of a non-RSV
3 molecule selected from a cytokine, a T-helper epitope, a

4 restriction site marker, or a protein of a microbial pathogen
5 capable of eliciting a protective immune response in a mammalian
6 host.

1 36. The RSV of claim 26, which further comprises a
2 gene or gene from PIV replacing a corresponding gene or gene
3 segment of RSV.

1 37. The RSV of claim 36, wherein the PIV gene or gene
2 segment encodes HN or F glycoprotein of PIV1, PIV2, or PIV3.

1 38. An isolated infectious RSV particle which
2 comprises a recombinant RSV genome or antigenome, a major
3 nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a
4 large (L) polymerase protein, and a RNA polymerase elongation
5 factor, wherein the RSV genome or antigenome is modified by a
6 termination codon introduced within a selected gene, or by a
7 change in sequence, position, or presence of a GS or GE
8 transcription signal relative to the selected gene.

1 39. A method for stimulating the immune system of an
2 individual to induce protection against respiratory syncytial
3 virus, which comprises administering to the individual an
4 immunologically sufficient amount of the isolated attenuated
5 recombinant RSV of claim 1, 26 or 38 in a physiologically
6 acceptable carrier.

1 40. The method of claim 39, administered in a dose of
2 10^3 to 10^6 PFU of the attenuated RSV.

1 41. The method of claim 39, wherein the attenuated
2 RSV is administered to the upper respiratory tract.

1 42. The method of claim 39, wherein the attenuated
2 RSV is administered by spray, droplet or aerosol.

1 43. The method of claim 39, wherein the attenuated
2 RSV is administered to an individual seronegative for antibodies

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1 60. The method of Claim 51, wherein the RSV genome or
2 antigenome further comprises a PIV gene or gene segment encoding
3 HN or F glycoprotein of PIV1, PIV2, or PIV3.

61. An RSV strain selected from *cpts* RSV 248 (ATCC VR 2450), *cpts* 248/404 (ATCC VR 2454), *cpts* 248/955 (ATCC VR 2453), *cpts* RSV 530 (ATCC VR 2452), *cpts* 530/1009 (ATCC VR 2451), or *cpts* 530/1030 (ATCC VR 2455).

1 62. An RSV strain selected from B-1 cp52/2B5 (ATCC VR
2 2542) or B-1 cp-23 (ATCC VR).

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3 to RSV or possessing transplacentally acquired maternal
4 antibodies to RSV.

1 44. A vaccine to induce protection against RSV, which
2 comprises an immunologically sufficient amount of the isolated
3 attenuated recombinant RSV of claim 1, 26 or 38 in a
4 physiologically acceptable carrier.

1 45. The vaccine of claim 44, formulated in a dose of
2 10^3 to 10^6 PFU of the attenuated RSV.

1 46. The vaccine of claim 44, formulated for
2 administration to the upper respiratory tract by spray, droplet
3 or aerosol.

1 47. A composition which comprises an expression
2 vector comprising an isolated polynucleotide molecule encoding a
3 RSV genome or antigenome having at least two attenuating
4 mutations, at least one of the mutations specifying a
5 temperature-sensitive substitution at amino acid Phe₅₂₁, Gln₈₃₁,
6 Met₁₁₆₉, or Tyr₁₃₂₁ in the RSV polymerase gene or a temperature-
7 sensitive nucleotide substitution in the gene-start sequence of
8 gene M2, and one or more expression vector which comprises one
9 or more polynucleotide molecules encoding N, P, L and RNA
10 polymerase elongation factor proteins of RSV, whereby upon
11 expression an infectious RSV particle is produced.

1 ¹³
~~48.~~ The composition of claim ¹²~~47~~, wherein the
2 infectious RSV particle is a virus.

1 ¹⁴
~~49.~~ A method for producing an infectious attenuated
2 RSV particle from one or more isolated polynucleotide molecules
3 encoding said RSV, comprising:

4 coexpressing in a cell or cell-free lysate the
5 expression vectors of claim ¹²~~47~~, thereby producing said
6 infectious RSV particle.

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1 50. The method of claim 49, wherein the RSV genome or
2 antigenome and the N, P, L and RNA polymerase elongation factor
3 proteins are expressed by two or more different expression
4 vectors.

1 51. The method of claim 49, wherein the RSV wherein
2 the genome or antigenome is modified to ablate or modulate
3 expression of a SH, NS1, NS2 or G gene or a cis-acting
4 regulatory sequence.

1 52. The method of claim 51, wherein the SH gene is
2 deleted.

1 53. The method of claim 51, wherein the NS2 gene is
2 reciprocally substituted in position for the SH gene.

1 54. The method of claim 51, wherein the cis-acting
2 regulatory sequence is replaced by a heterologous regulatory
3 sequence.

1 55. The method of claim 54, wherein the heterologous
2 regulatory sequence is a cis-acting regulatory sequence of a
3 different RSV gene or of PIV.

1 56. The method of claim 51, wherein the RSV genome or
2 antigenome further comprises a gene or gene segment encoding an
3 immunogenic F or G protein region of a different RSV subgroup.

1 57. The method of claim 51, wherein the genome or
2 antigenome comprises a chimera of a human RSV sequence and at
3 least one non-human RSV sequence.

1 58. The method of claim 51, wherein the genome or
2 antigenome encodes a human RSV in which a selected gene or gene
3 segment is replaced with a counterpart gene or gene segment from
4 a heterologous RSV.

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